

Phosphorylation of Synaptic GTPase Activating Protein (synGAP) by Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) and cyclin-dependent kinase 5 (CDK5) alters the ratio of its GAP activity toward Ras and Rap GTPases\*

**<sup>1</sup>Ward G. Walkup IV, <sup>1,S</sup>Lorraine Washburn, <sup>2</sup>Michael J. Sweredoski, <sup>1,#</sup>Holly J. Carlisle, <sup>2,†</sup>Robert L. Graham, <sup>2</sup>Sonja Hess, and <sup>1</sup>Mary B. Kennedy**

From the <sup>1</sup>Division of Biology and Biological Engineering, and the <sup>2</sup>Proteome Exploration Laboratory of the Beckman Institute, California Institute of Technology, Pasadena, CA 91125

\*Running title: *Distinct regulation of Ras and Rap GAP activities of synGAP*

To whom correspondence should be addressed: Mary B. Kennedy, Division of Biology and Biological Engineering 216-76, California Institute of Technology, Pasadena, CA, USA 91125  
Tel.: (626) 395-3924; Fax: (626) 395-8474; Email: [kennedym@its.caltech.edu](mailto:kennedym@its.caltech.edu)

SUPPLEMENTARY TABLE 1. *CaMKII and CDK5 Phosphorylation Sites in R-synGAP*

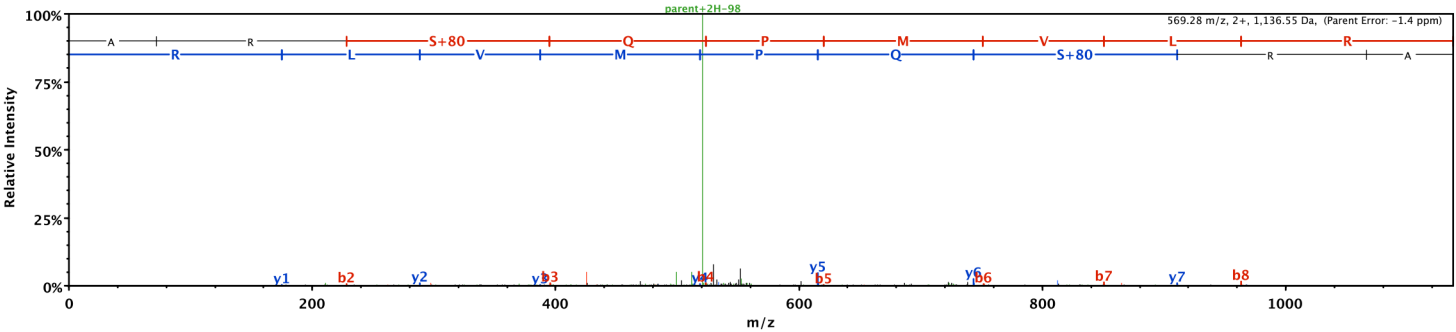
CaMKII and CDK5 phosphorylation sites in r-synGAP detected by mass spectrometry as described under methods. MS2 and MS3 spectra corresponding to each tryptic peptide are contained in the accompanying pdf file. When several unique peptides were detected for a single phosphorylation site, we have shown only the peptide with the lowest predicted false localization rate.

Phosphorylated Residue	Kinase	Peptide Sequence (Modified Residue(s) in Lower Case)	Mascot Ion Score	Mascot Delta Score	False Localization Rate
S728	CDK5	(R)ARsQPMVLR(G)	42.6	42.6	0.0%
S773	CDK5	(R)LPsPTKEKPPPPPGGGK(D)	55.6	14.6	0.4%
T775	CDK5	(R)GLNSSmDMARLPSPtK(E)	51.8	9.8	1.4%
S802	CDK5	(R)SsPAYcTSSSDITEPEQK(M)	84.5	0	>17%
S842	CDK5	(R)LNSSSVSNLAAVGDLLHSSQASLTAALGLRPAPAGR(L)	116.3	12.7	0.7%
S728	CaMKII	(R)ARsQPMVLR(G)	36	36	0.0%
S737	CaMKII	(R)GPsaEMQGYMMR(D)	70.9	70.9	0.0%
S750	CaMKII	(R)DLNsSIDLQSFmAR(G)	48.3	1.7	11.0%
S751	CaMKII	(R)DLNsSIDLQSFmAR(G)	83.7	17.2	0.2%
S756	CaMKII	(R)DLNsSIDLQsFmAR(G)	50.1	0	>17%
S764	CaMKII	(R)GLNsSMDMARLPSPtK(E)	55.7	2.2	9.5%
S765	CaMKII	(R)GLNsSMDMAR(L)	52.5	14	0.5%

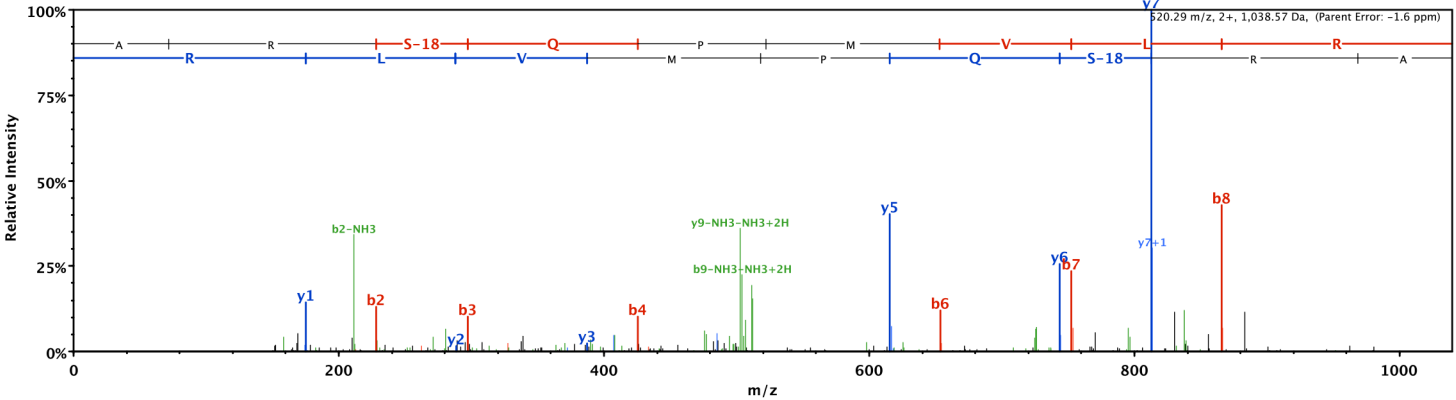
Phosphorylated Residue	Kinase	Peptide Sequence (Modified Residue(s) in Lower Case)	Mascot Ion Score	Mascot Delta Score	False Localization Rate
S808	CaMKII	(R)SSPAYcTsSSDITEPEQK(M)	44.2	3.7	6.4%
S809	CaMKII	(R)SSPAYcTSsSDITEPEQK(M)	47.5	5.6	4.0%
S810	CaMKII	(R)SSPAYcTSSsDITEPEQK(M)	46.9	0	>17%
S821	CaMKII	(K)MLsVNKSVSMLDLQGDGPGR(L)	66.4	13.9	0.5%
S825	CaMKII	(K)mLSVNKsVSMLDLQGDGPGR(L)	85	25.2	0.0%
S827	CaMKII	(K)SVsMLDLQGDGPGR(L)	92.6	22	0.1%
S842	CaMKII	(R)LNsSSVSNLAAGVDLLHSSQASLTAAALGRPAPAGR(L)	84.1	10.2	1.2%
S843	CaMKII	(R)LNSsSVSNLAAGVDLLHSSQASLTAAALGRPAPAGR(L)	75	0	>17%
S882	CaMKII	(R)LSQSGSsSITAAGmR(L)	70.4	13	0.6%
S883	CaMKII	(R)LSQSGSsITAAGMR(L)	83	15	0.4%
T885	CaMKII	(R)LSQSGSSItAAGmR(L)	56.4	13.8	0.5%
S892	CaMKII	(R)LSQSGSSITAAGMRLsQmGVtTDGVPAQQLR(I)	44.5	18.4	0.2%
T897	CaMKII	(R)LSQMGVtTDGVPAQQLR(I)	92.6	18.4	0.2%
T898	CaMKII	(R)LSQmGVtTDGVPAQQLR(I)	85.1	0	>17%
S990	CaMKII	(K)SEDLSTGVKPPAASILHSHsYSDEFGPSGTDFTR(R)	41	6.8	2.9%
S1093	CaMKII	(R)QQsLsKEGSIGSGSGSGGGGGGGLKPSITK(Q)	32.9	13.6	0.5%
S1095	CaMKII	(R)QQsLsKEGSIGSGSGSGGGGGGGLKPSITK(Q)	32.9	13.6	0.5%
S1099	CaMKII	(K)EGsIGGsGGSGGGGGGGLKPSITK(Q)	42.6	37.6	0.0%
S1123	CaMKII	(K)QHsQTPSTLNPTmPASER(T)	73.7	33.9	0.0%
S1150	CaMKII	(R)TVAWVSNMPhLsADIESAHIEREEYK(L)	58	26.4	0.0%
S1171	CaMKII	(K)LKEYSKsMDESRLDR(V)	38.6	17.2	0.2%
S1210	CaMKII	(R)RLLsQEEQTSK(I)	54.3	39.8	0.0%
S1283	CaMKII	(R)GsFPPWVQQTRV(-)	74.1	69.9	0.0%
T1291	CaMKII	(R)LLDAQRGsFPPWVQQTRV(-)	31.5	31.5	0.0%

CaMKII S728

MS2

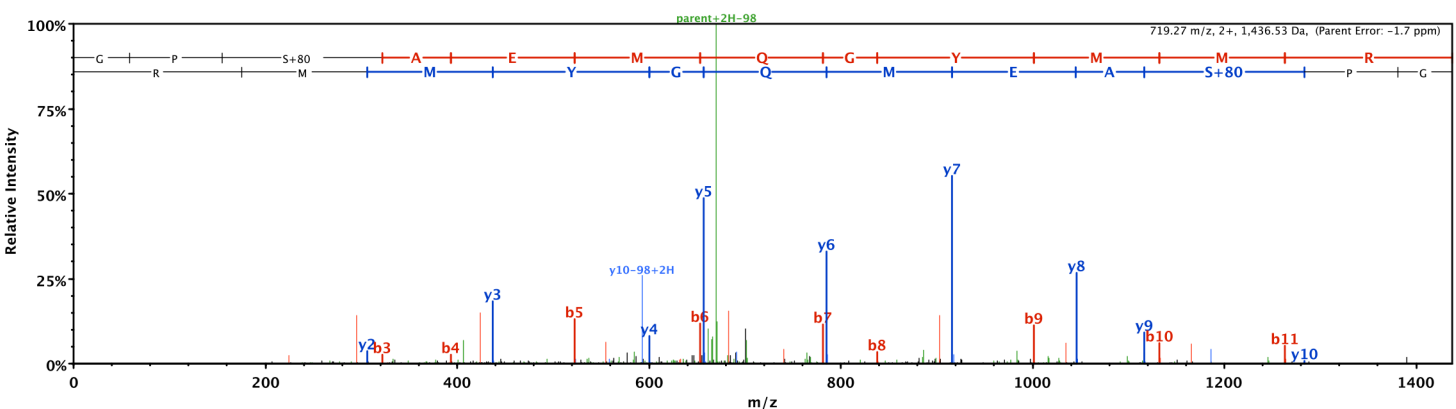


MS3

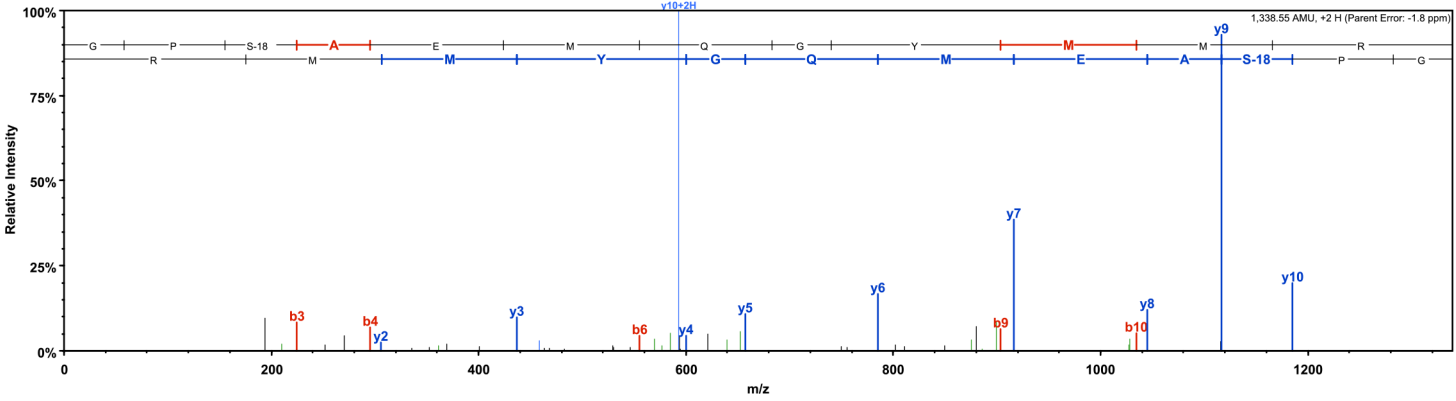


CaMKII S737

MS2

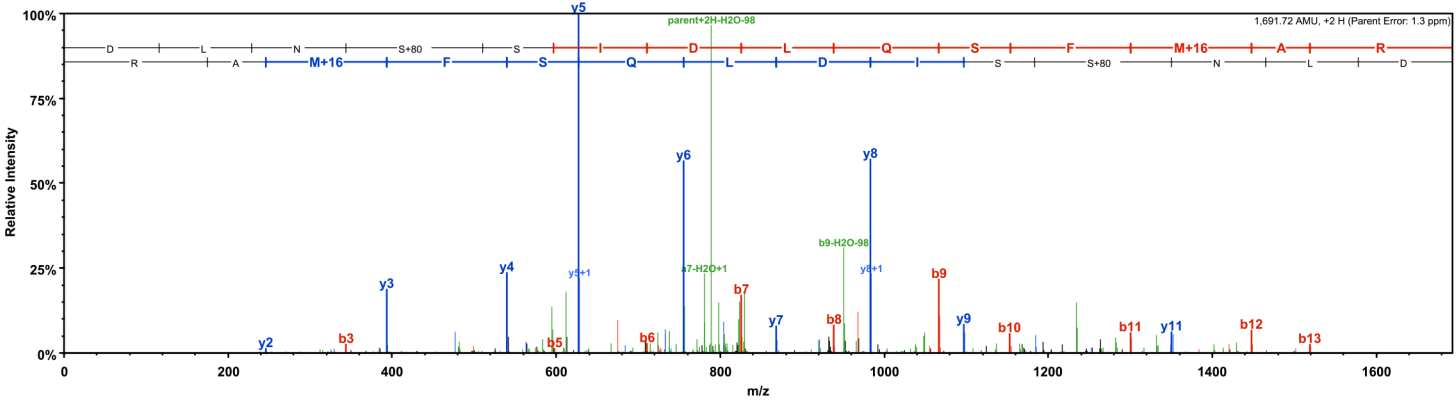


MS3



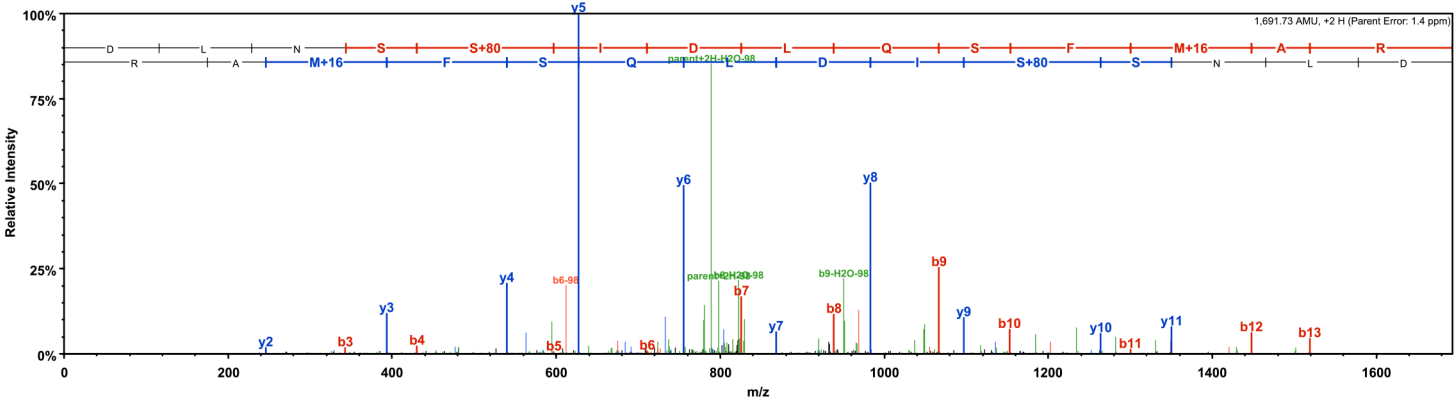
CaMKII S750

MS2



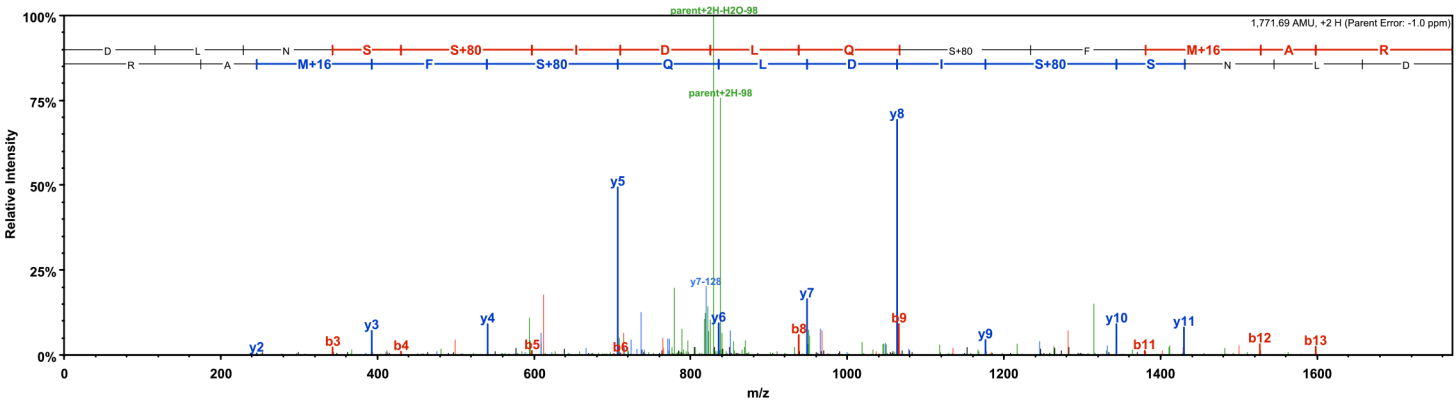
CaMKII S751

MS2



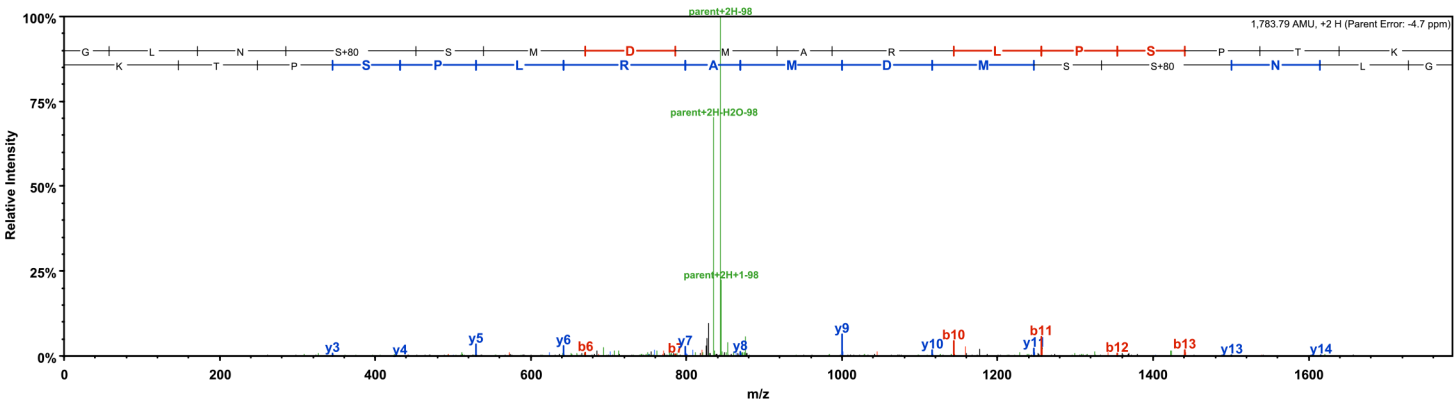
CaMKII S756

MS2



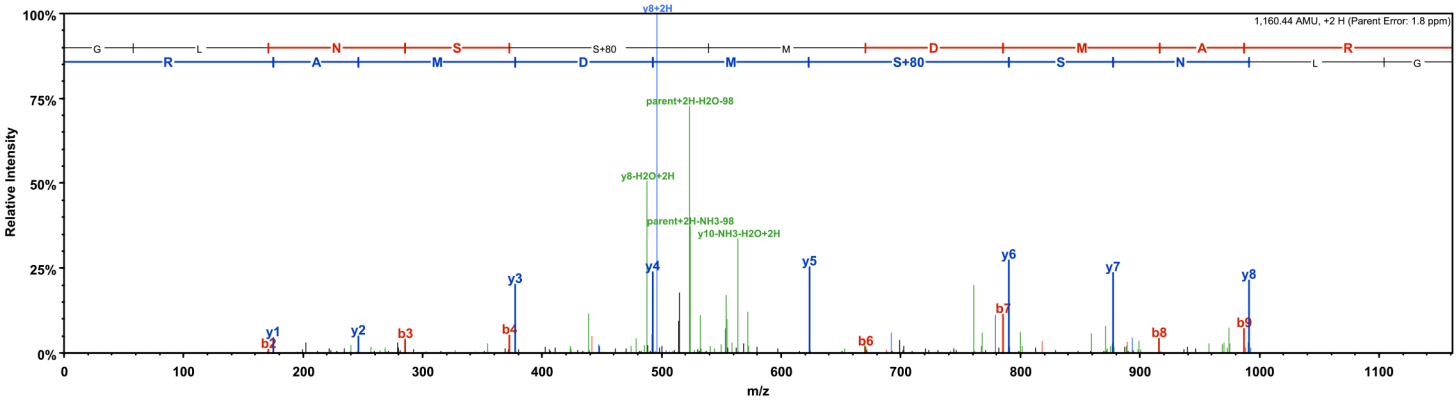
CaMKII S764

MS2

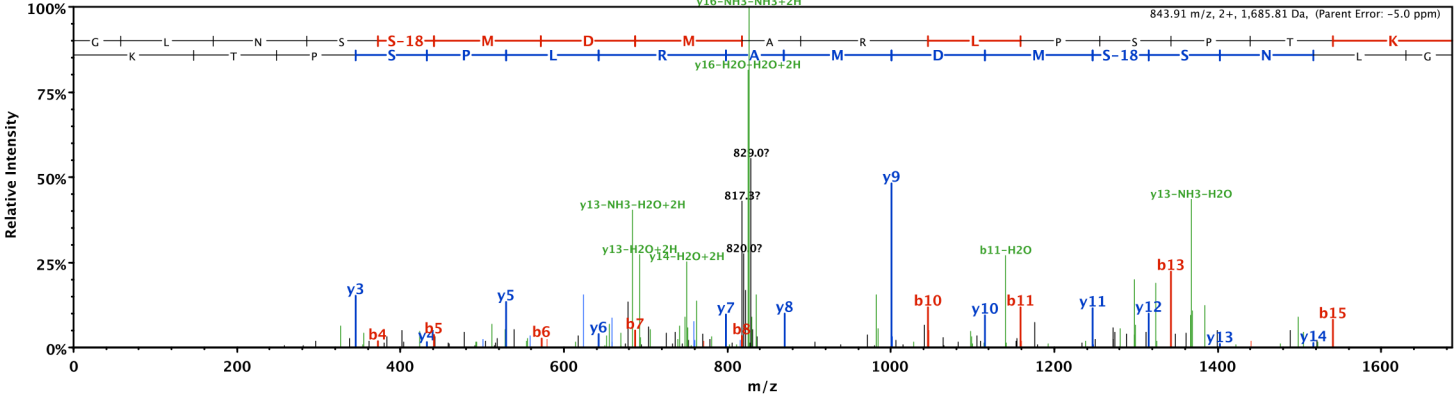


CaMKII S765

MS2

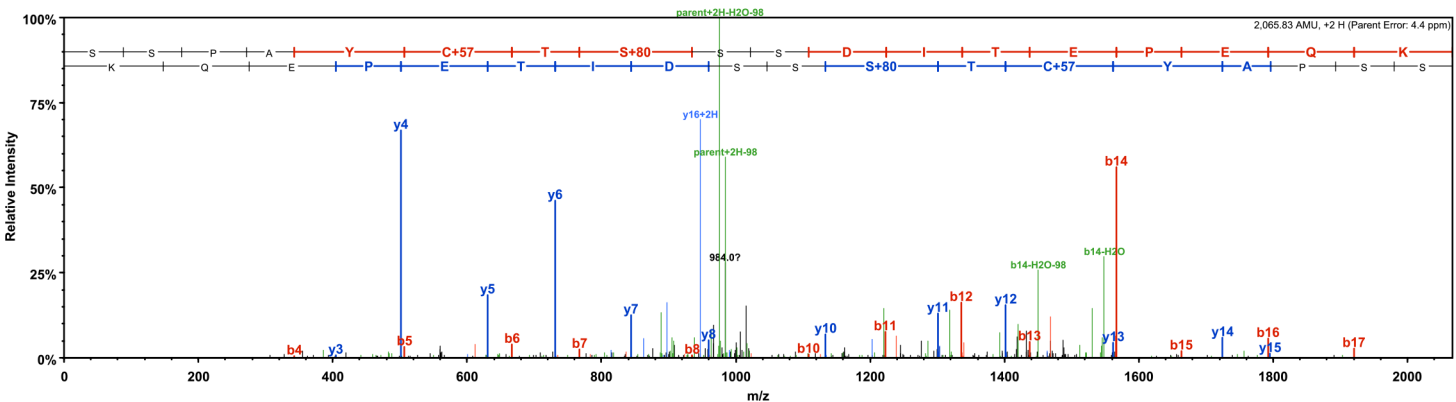


MS3



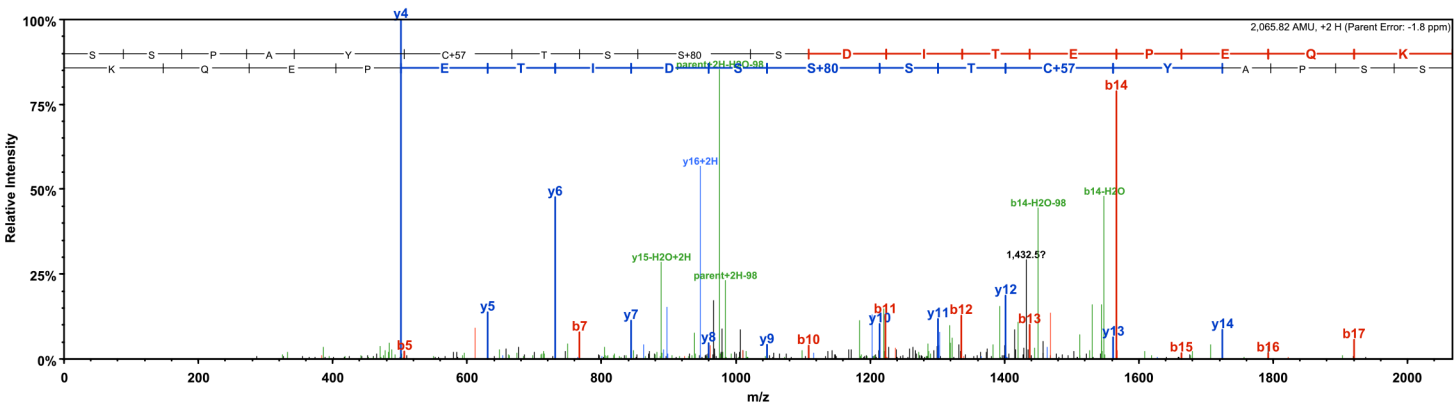
CaMKII S808

MS2



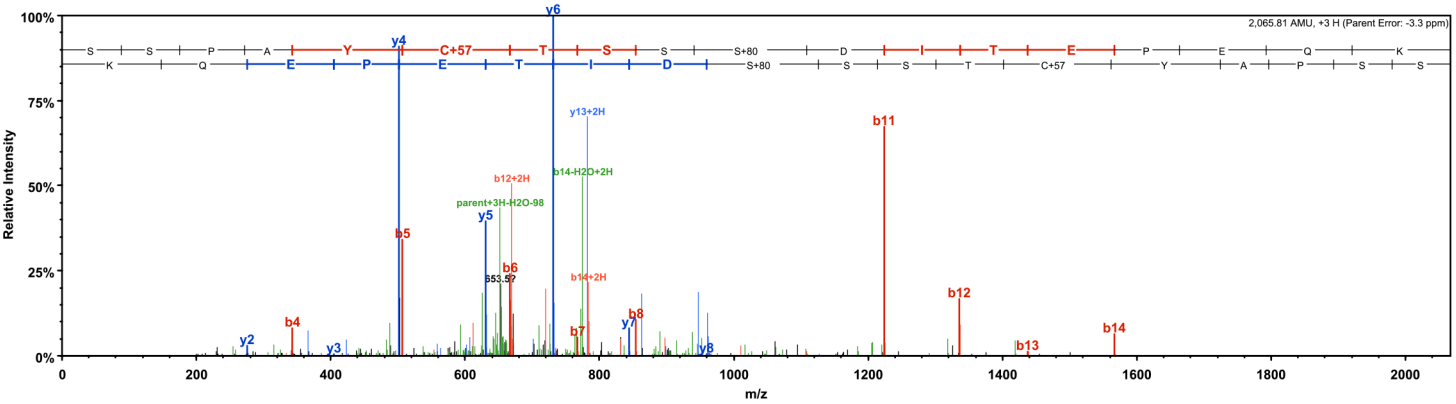
CaMKII S809

MS2



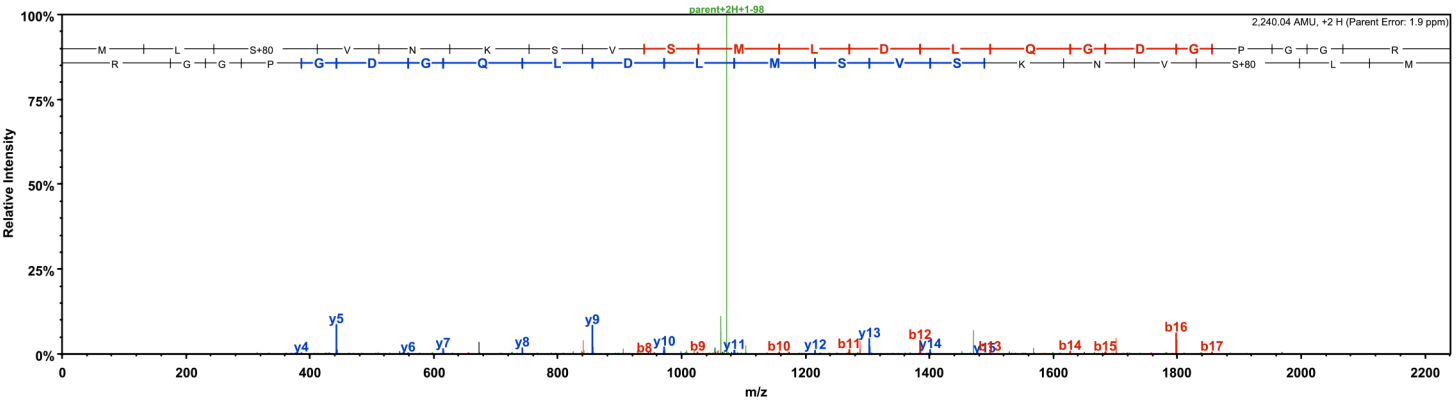
CaMKII S810

MS2



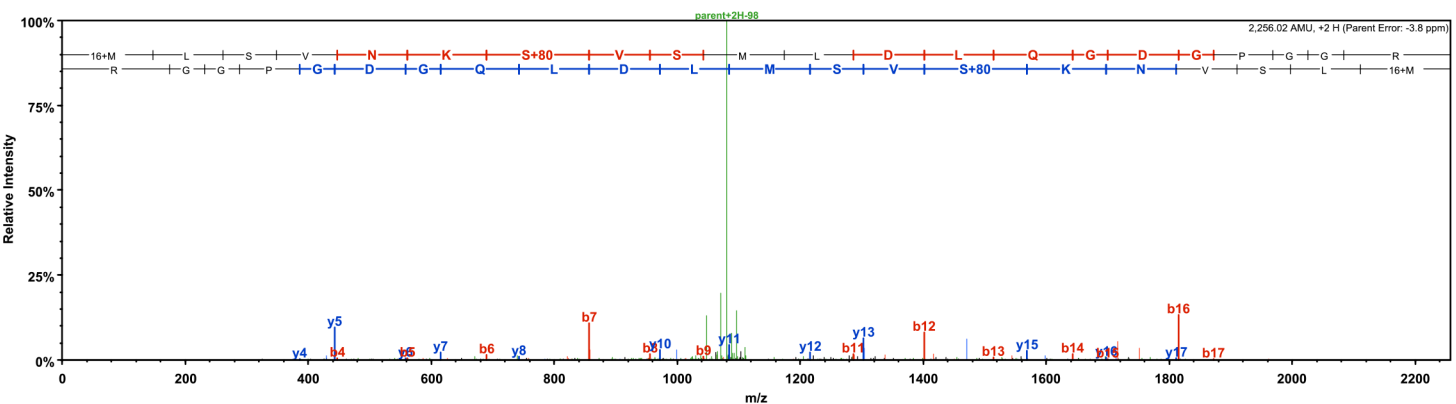
CaMKII S821

MS2

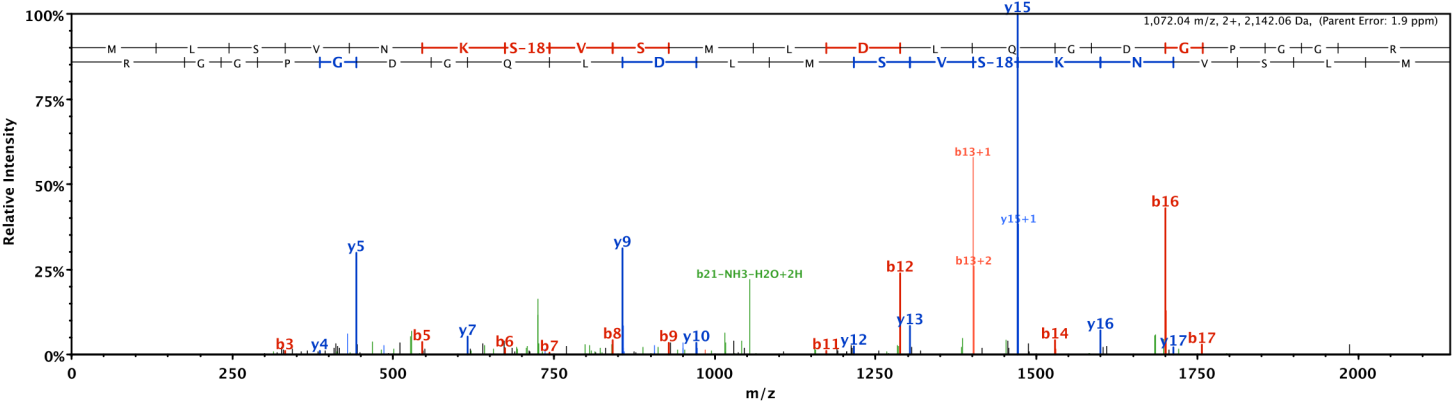


CaMKII S825

MS2

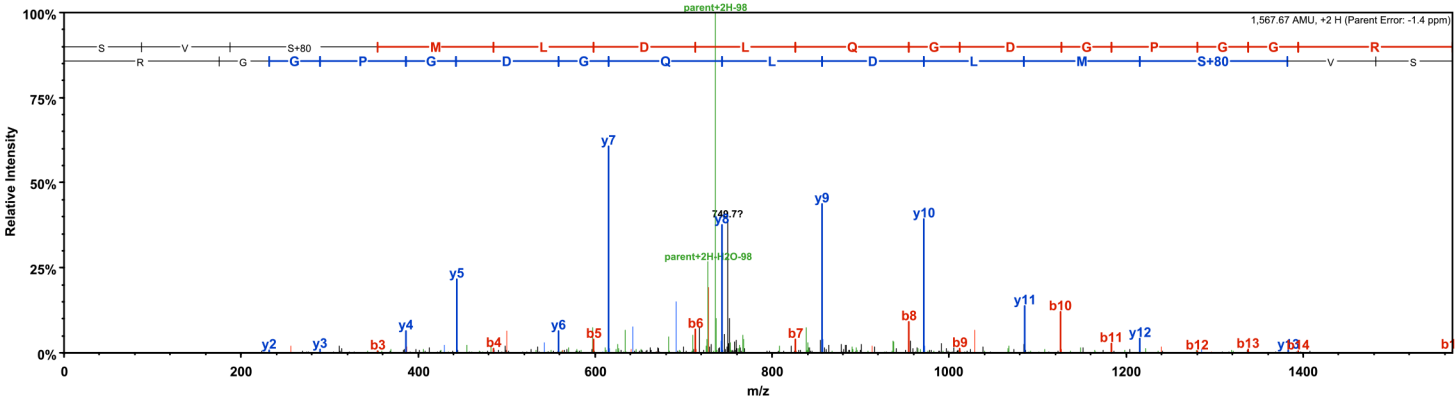


MS3

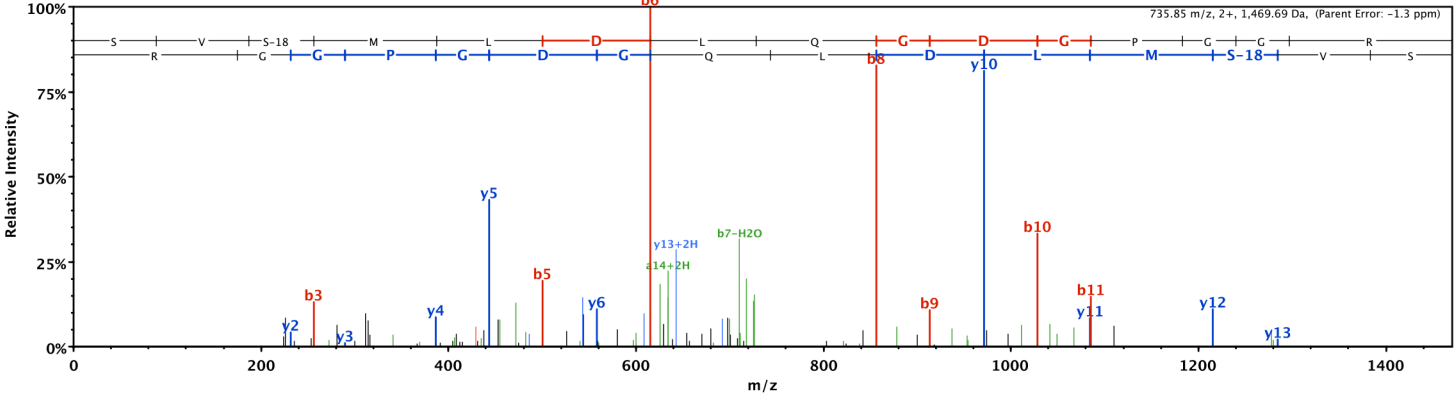


CaMKII S827

MS2

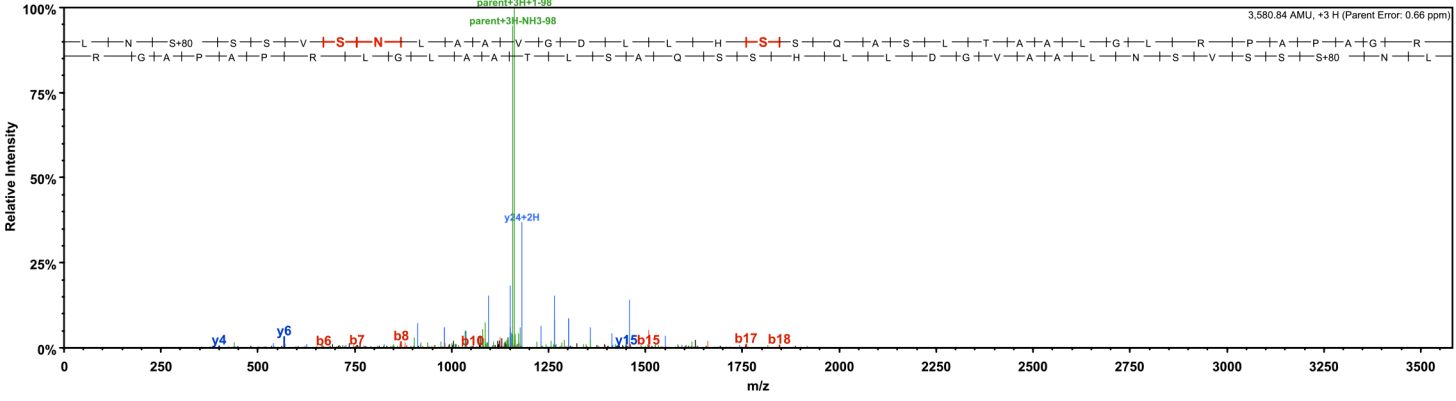


MS3



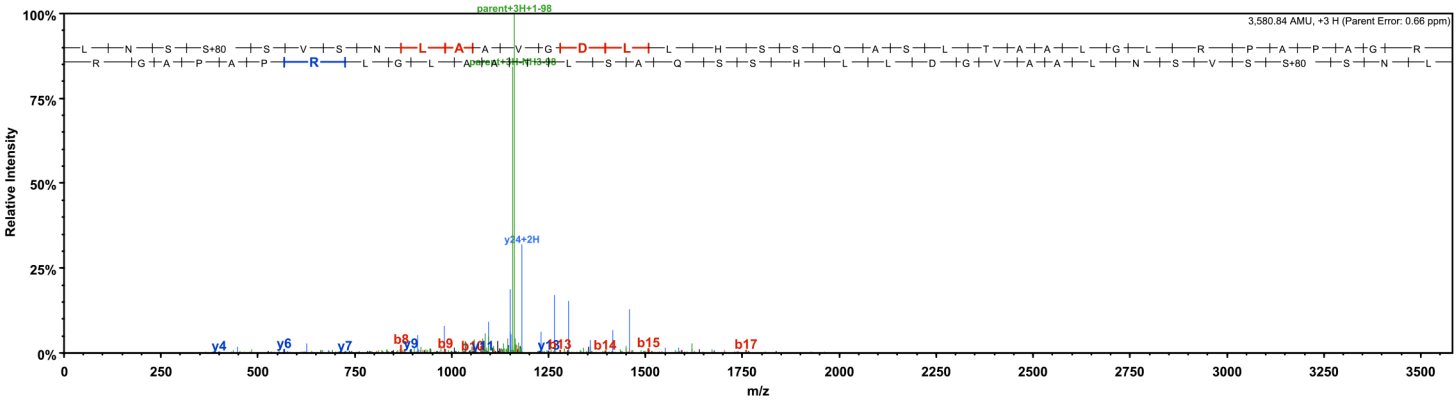
CaMKII S842

MS2



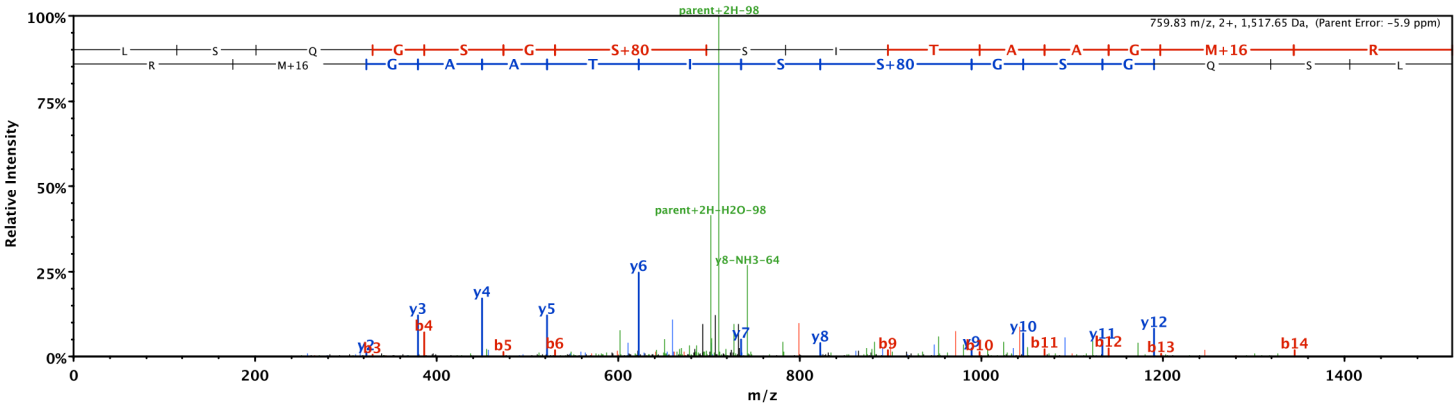
CaMKII S843

MS2

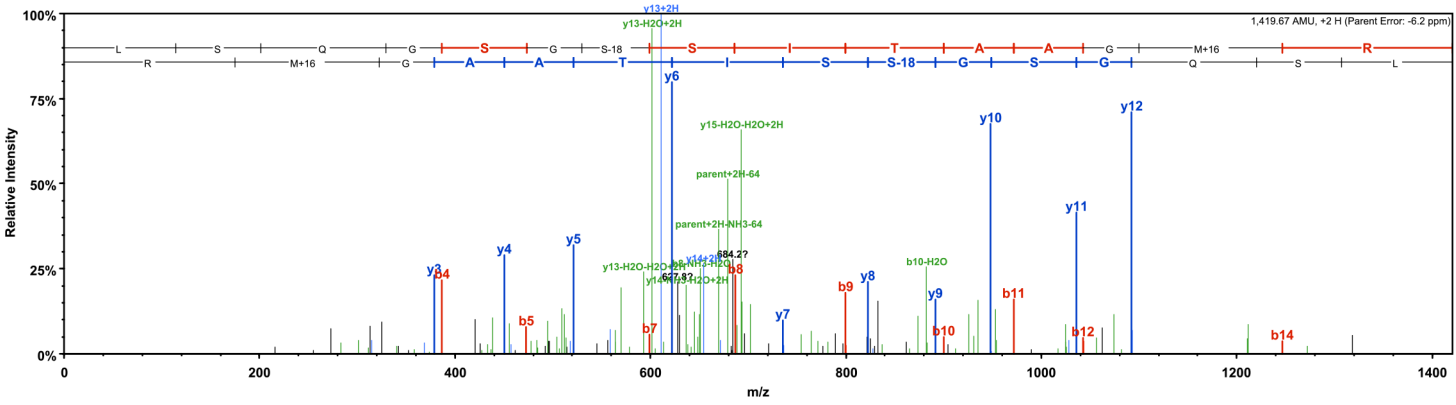


CaMKII S882

MS2

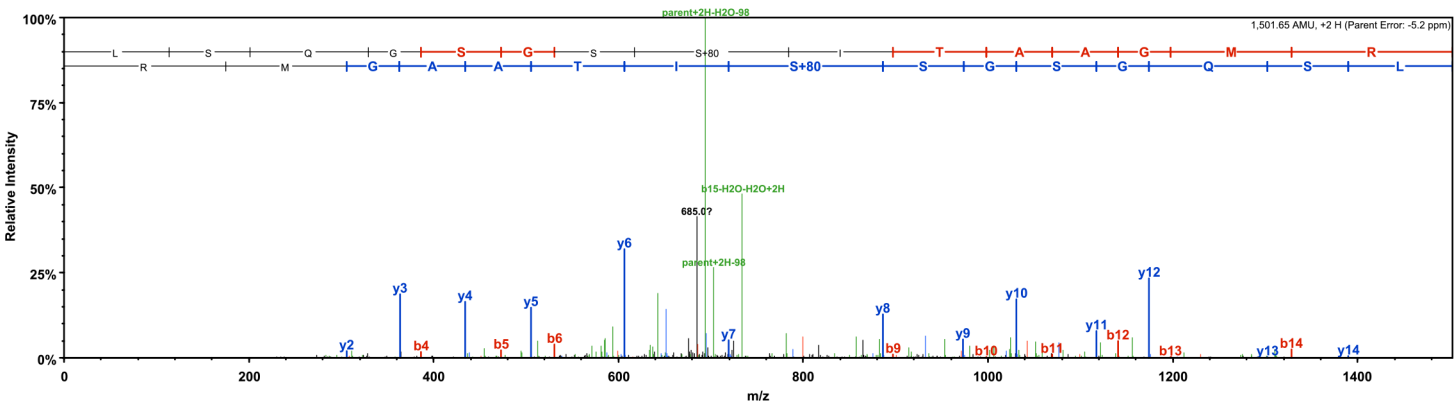


MS3

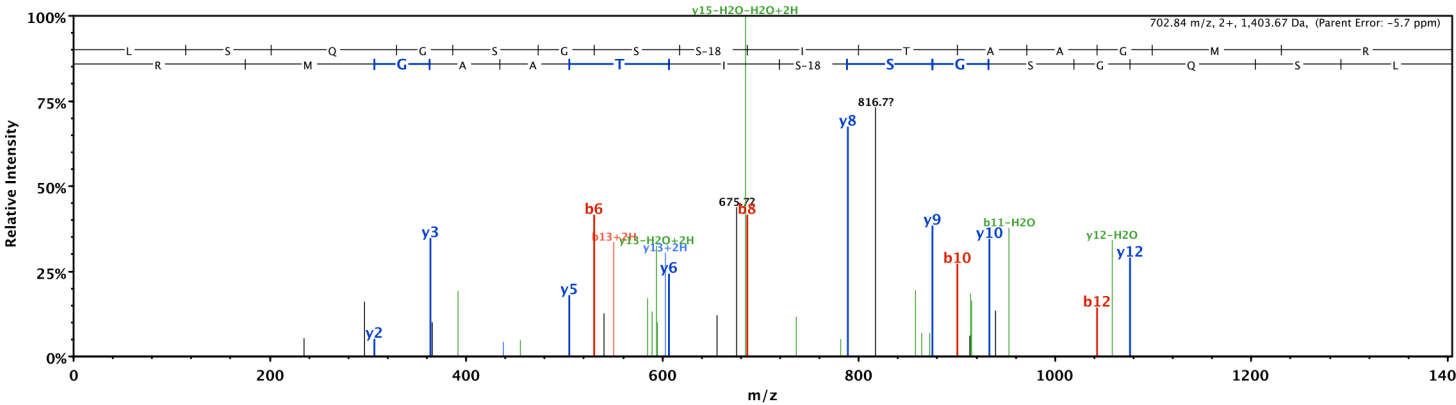


CaMKII S883

MS2



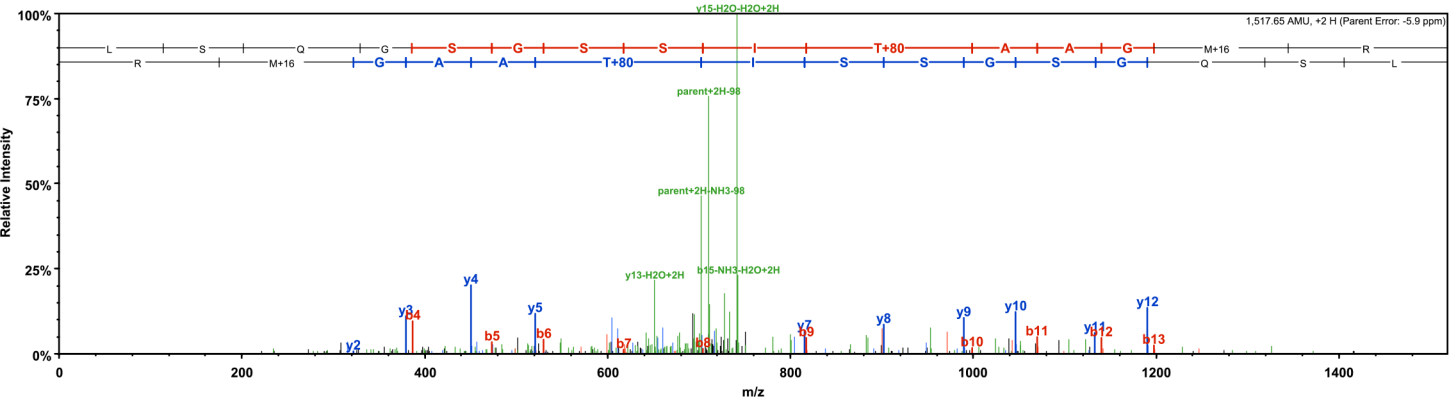
MS3





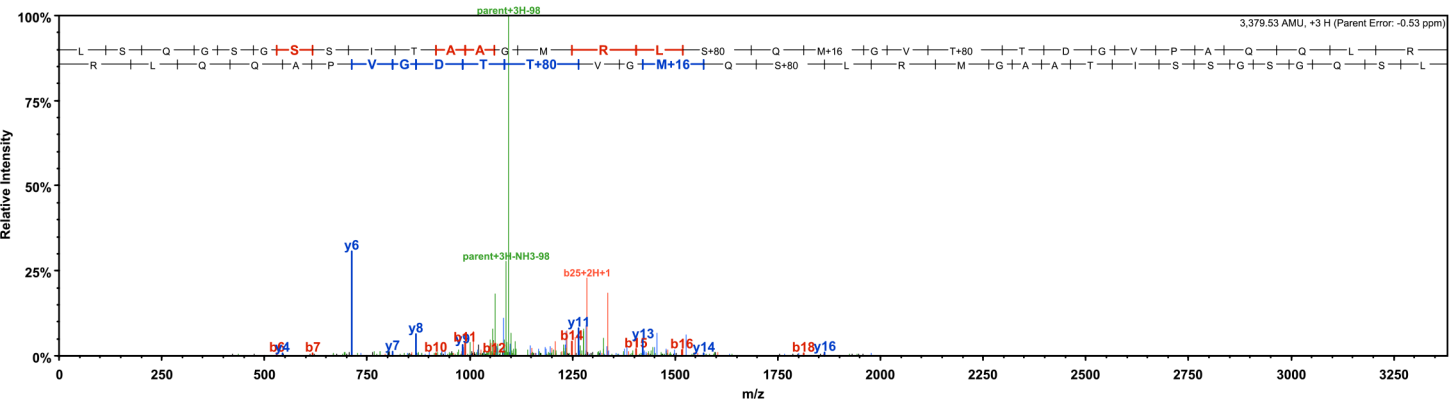
CaMKII T885

MS2



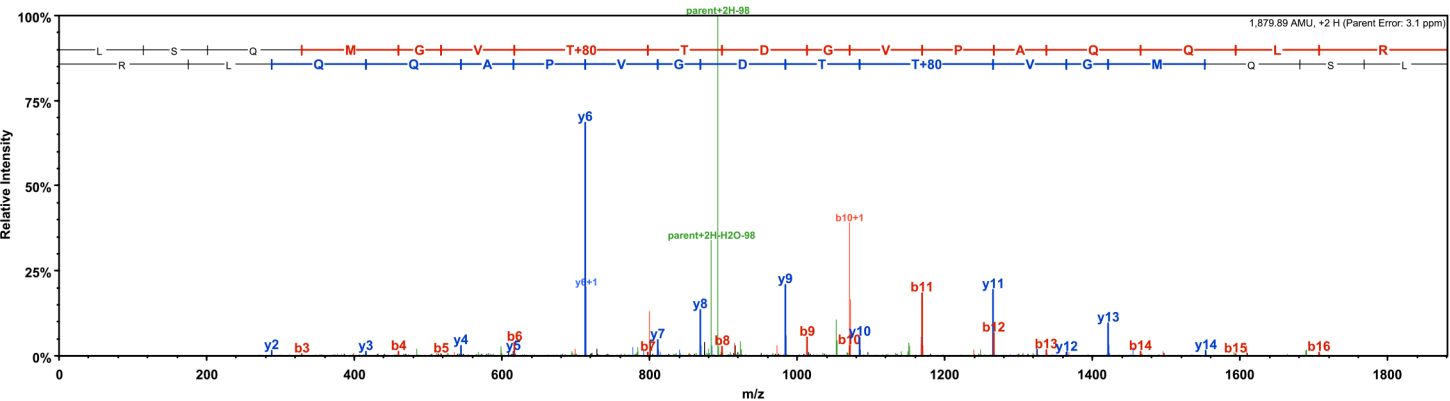
CaMKII S892

MS2

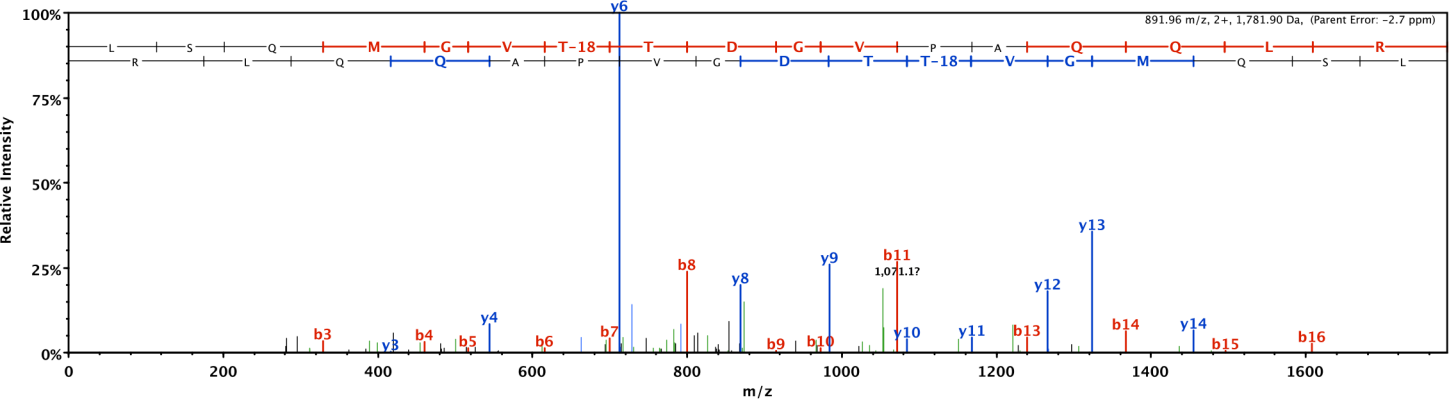


CaMKII T897

MS2

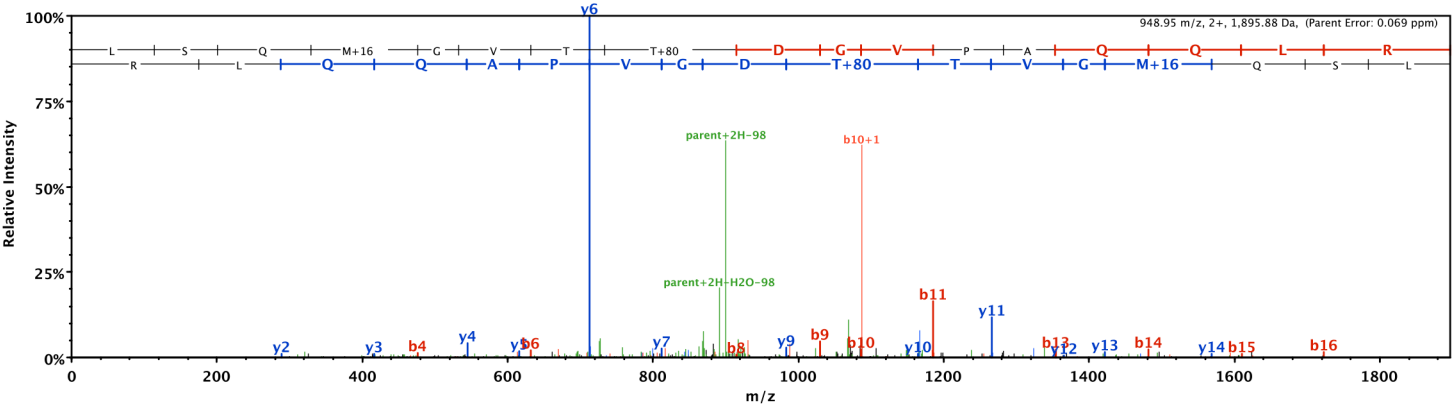


MS3

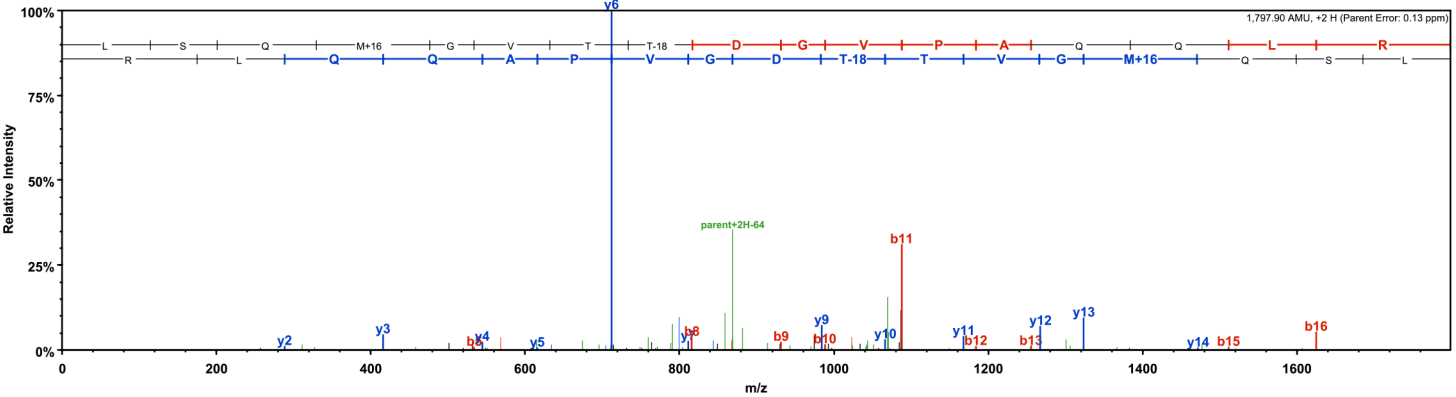


CaMKII T898

MS2

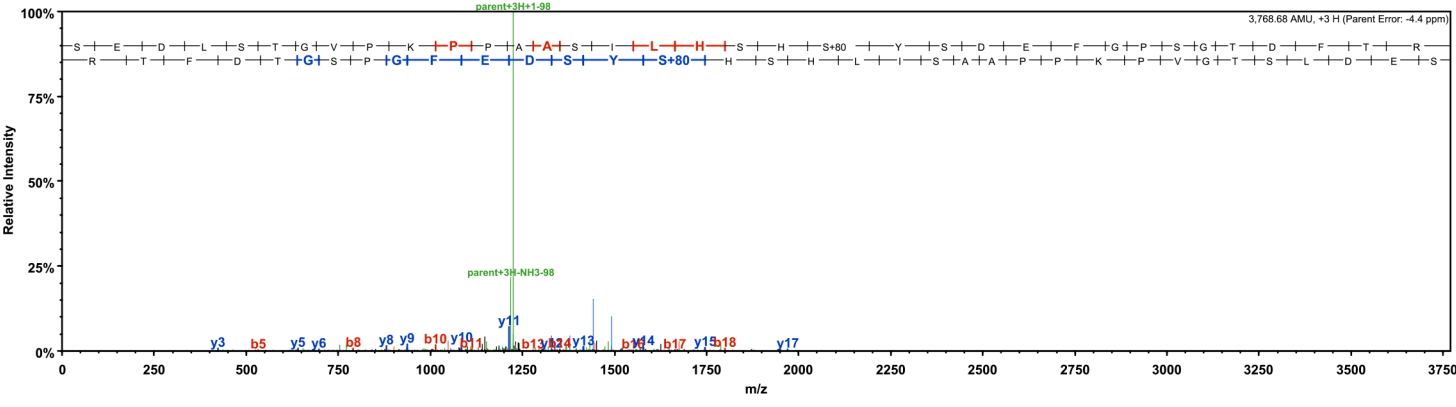


MS3

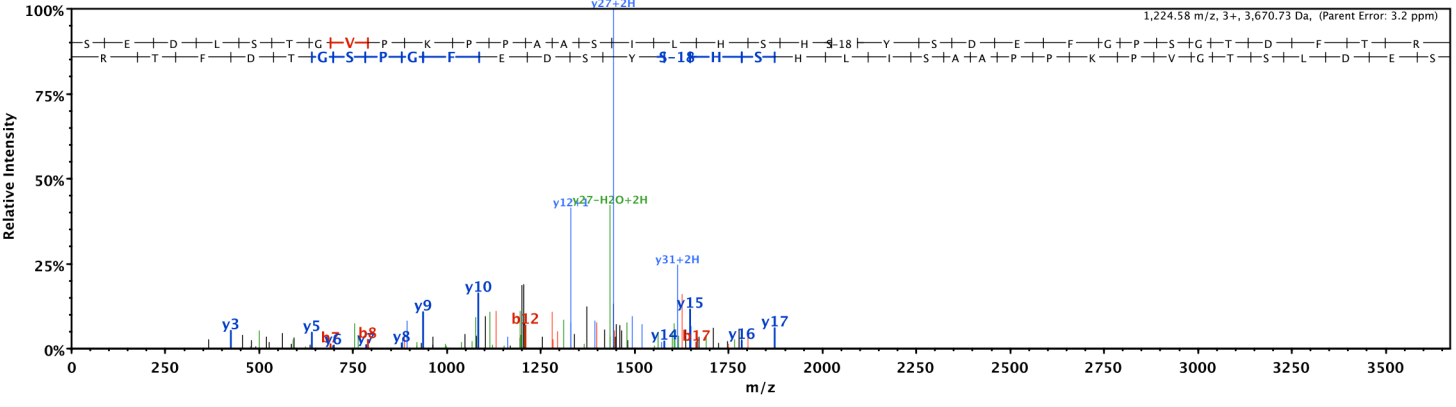


CaMKII S990

MS2

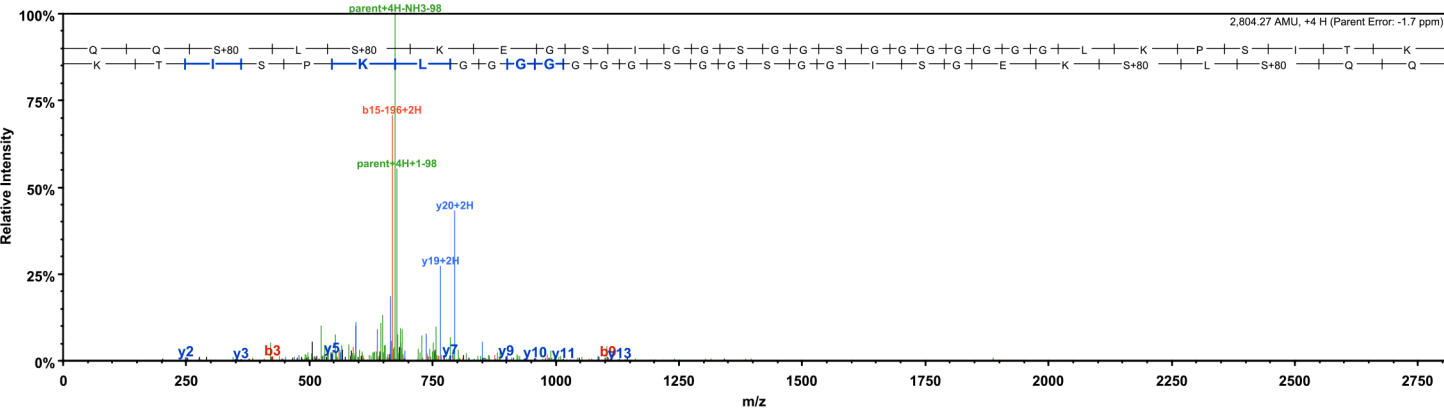


MS3



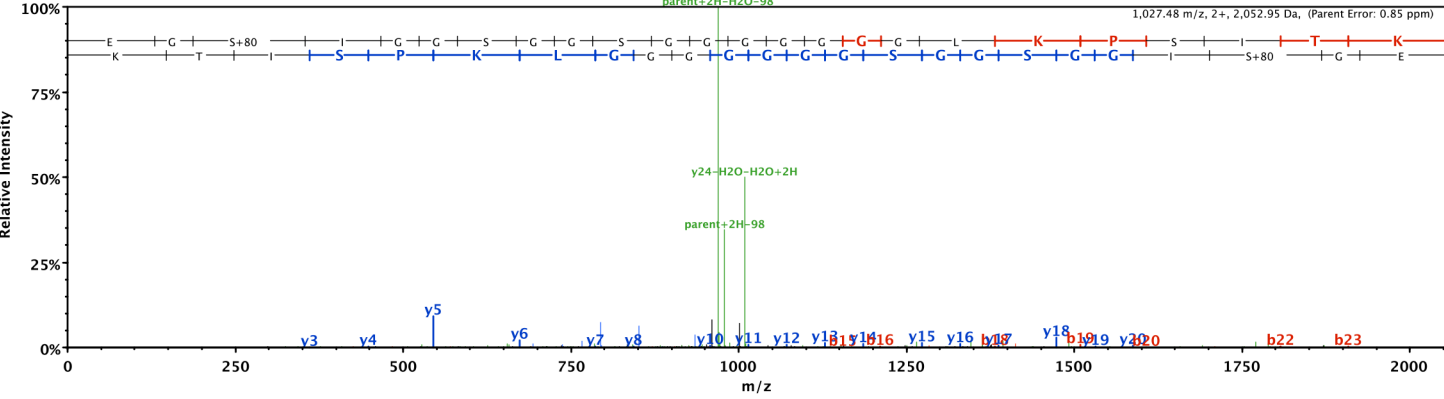
CaMKII S1093, S1095

MS2

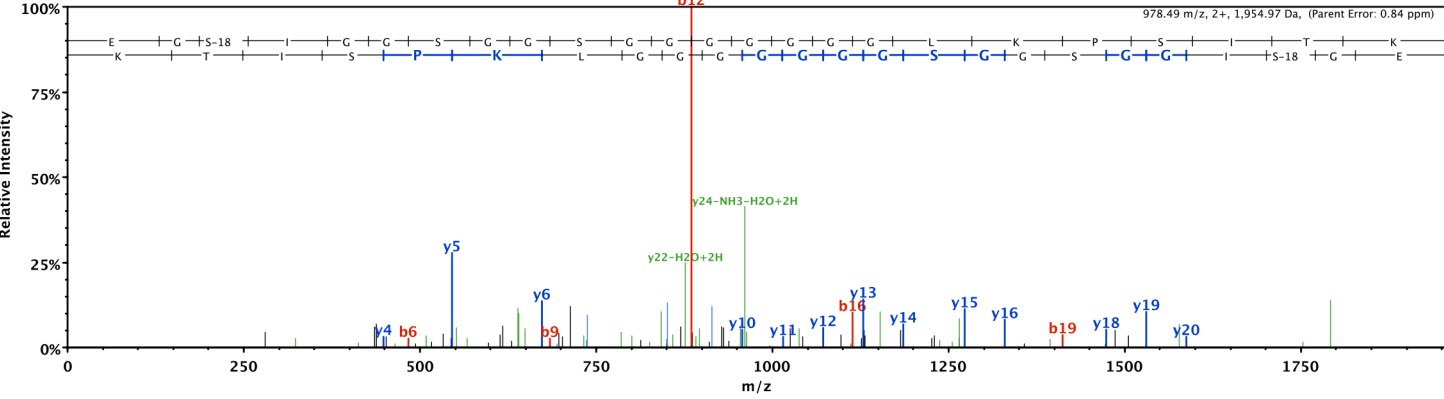


CaMKII S1099

MS2

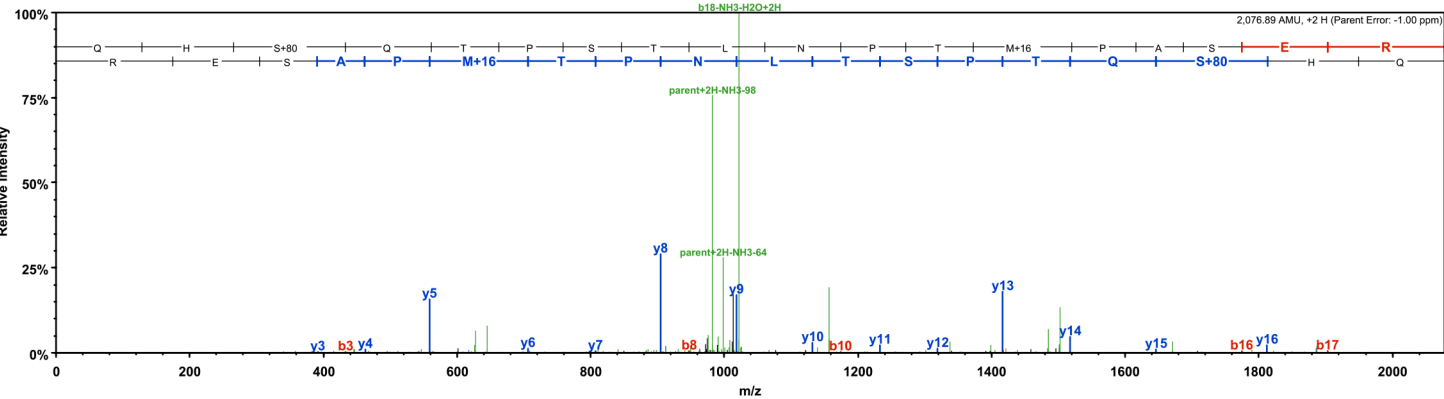


MS3



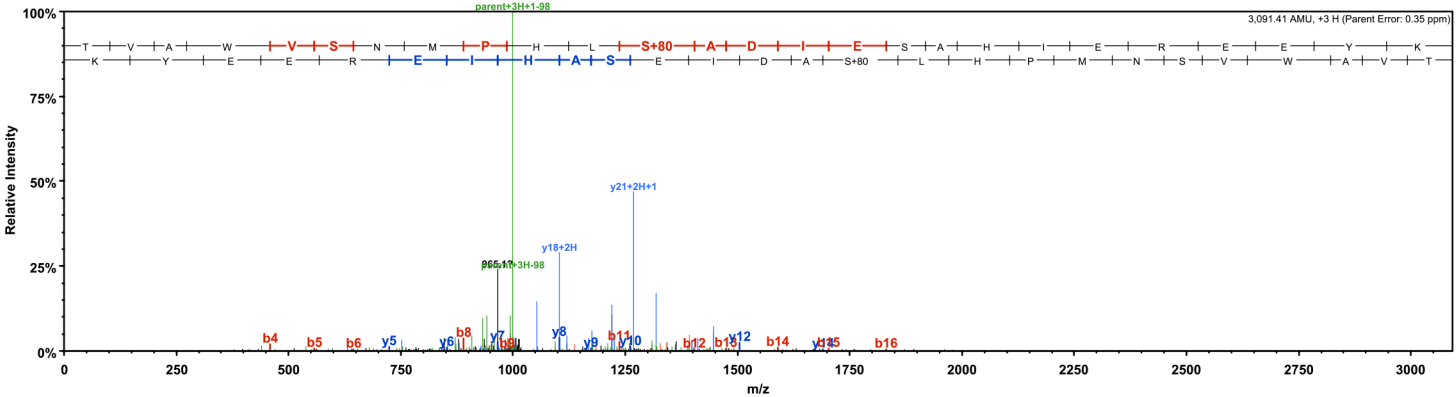
CaMKII S1123

MS2

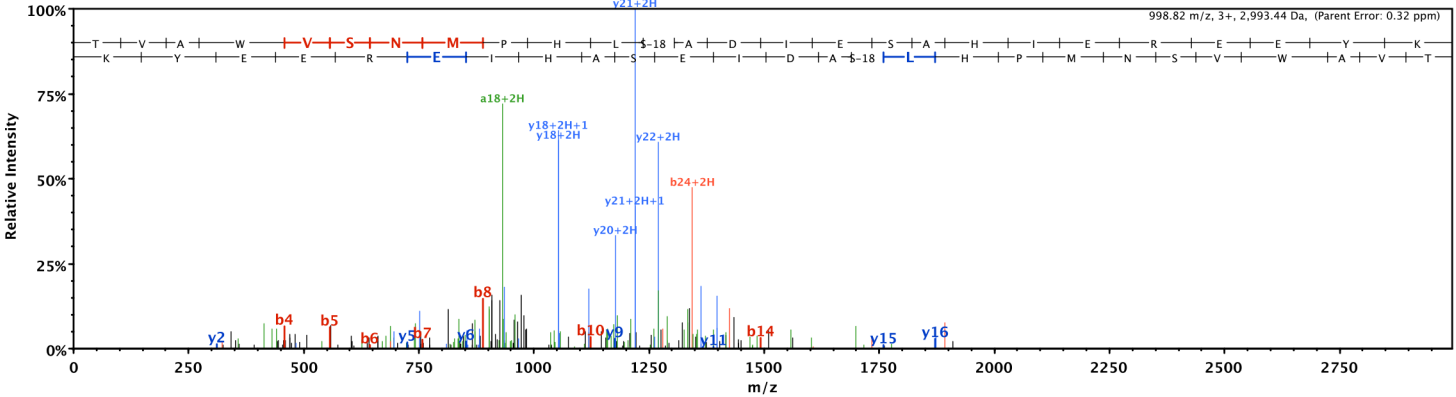


CaMKII S1150

MS2

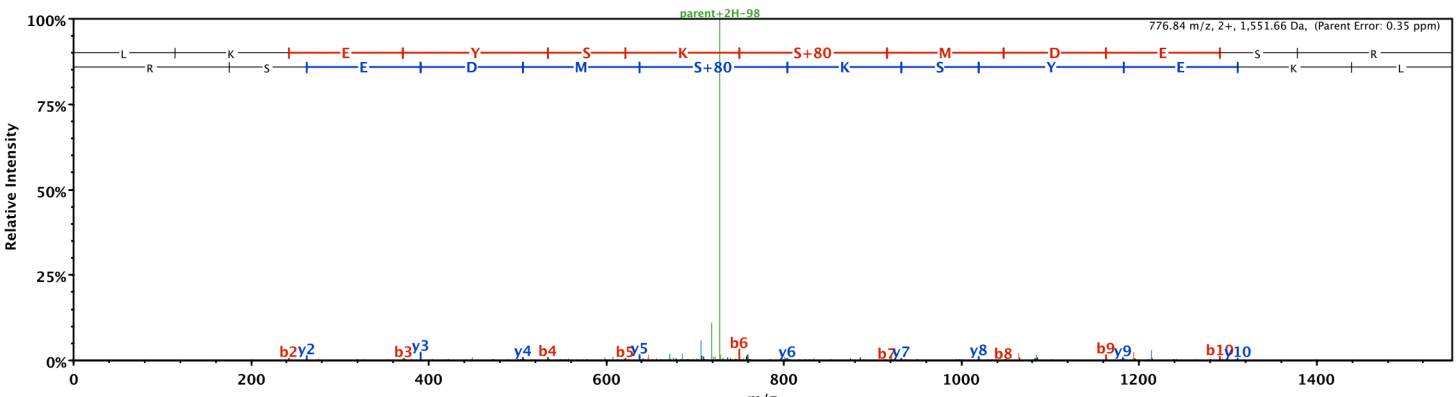


MS3

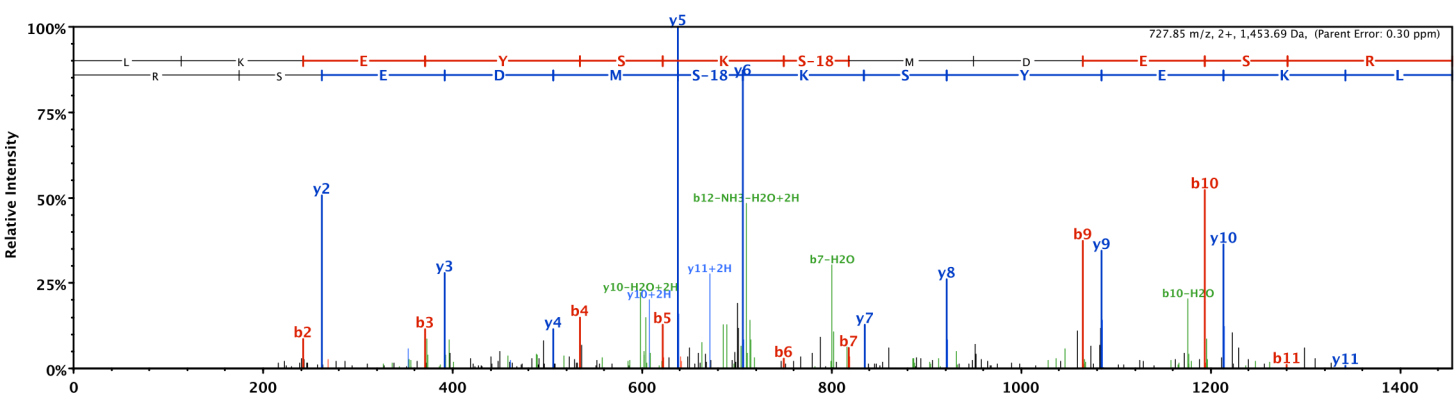


CaMKII S1171

MS2

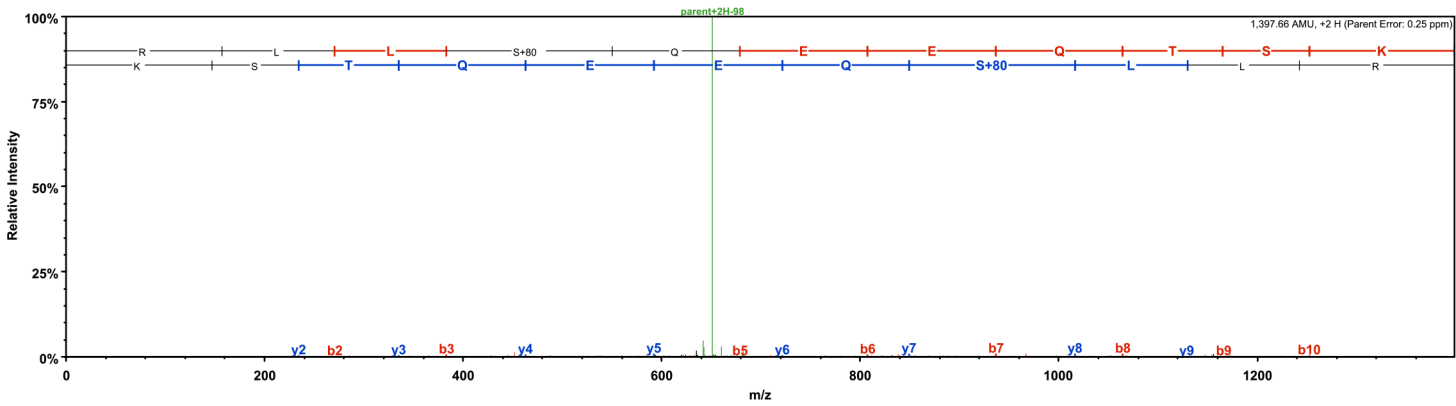


MS3

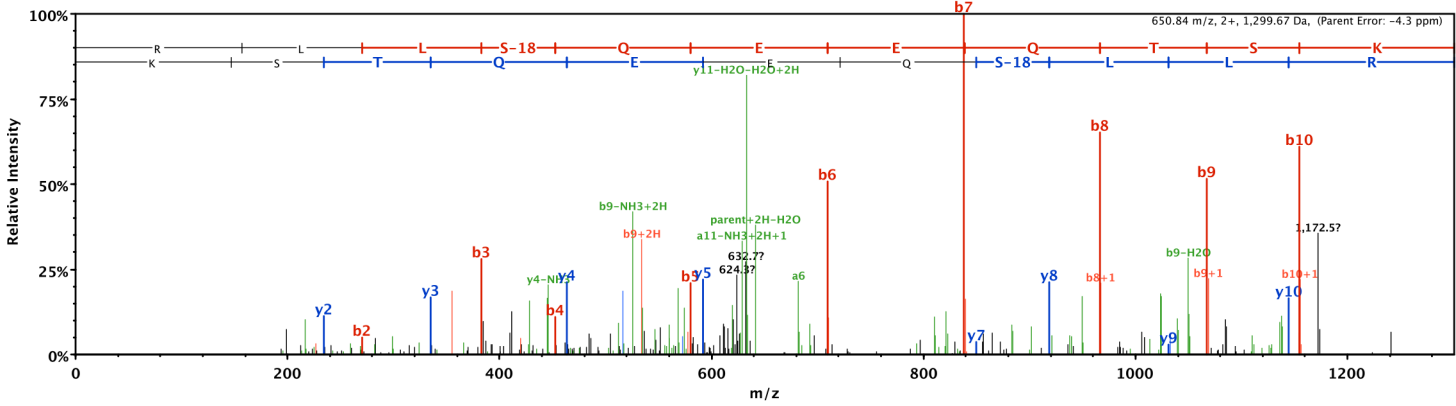


CaMKII S1210

MS2

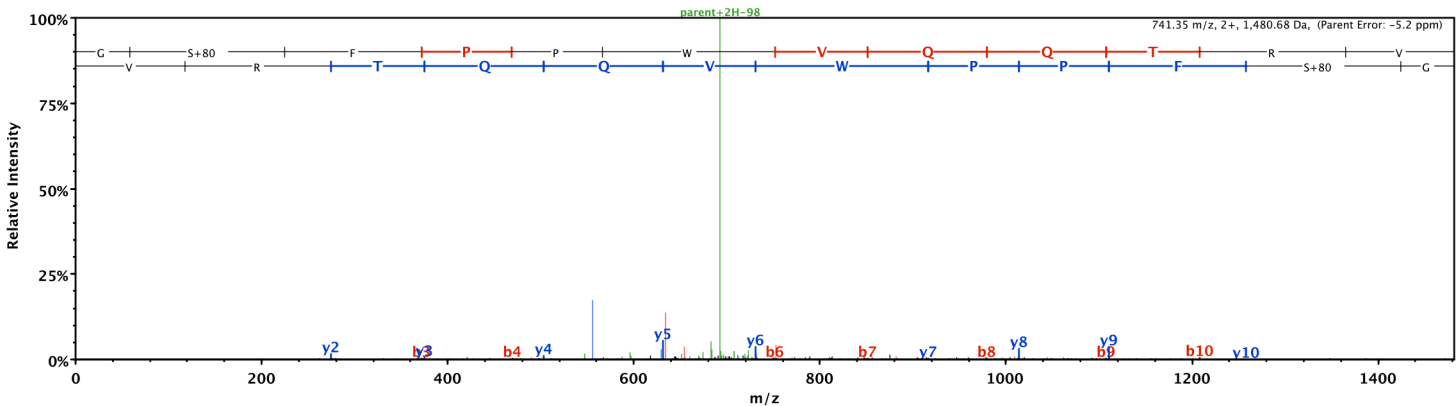


MS3

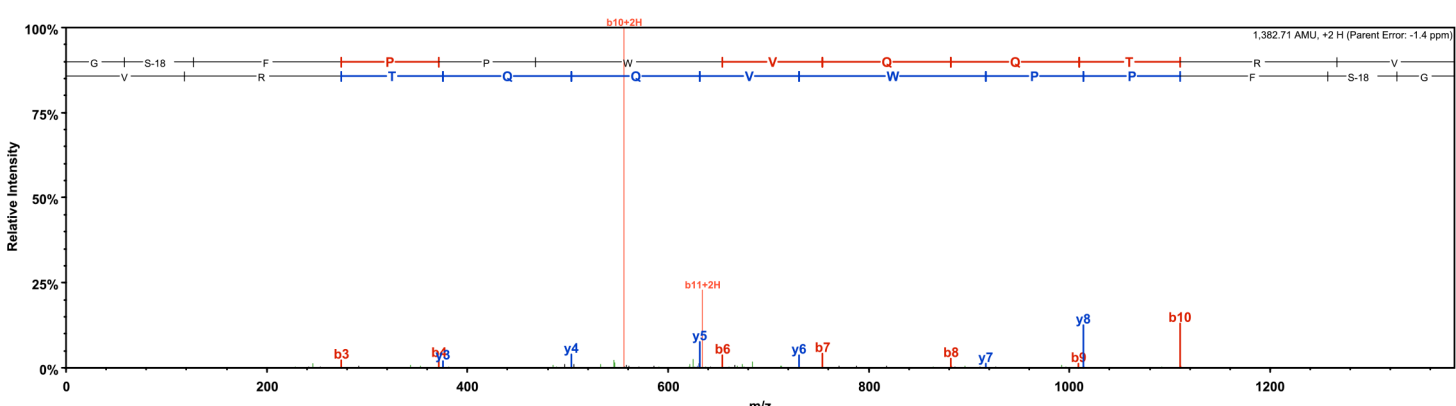


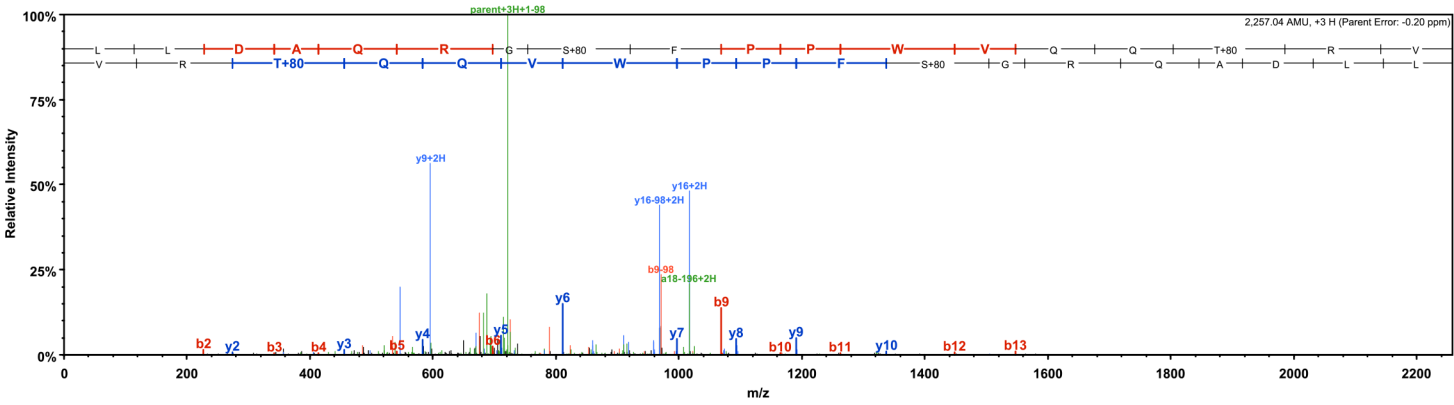
CaMKII S1283

MS2



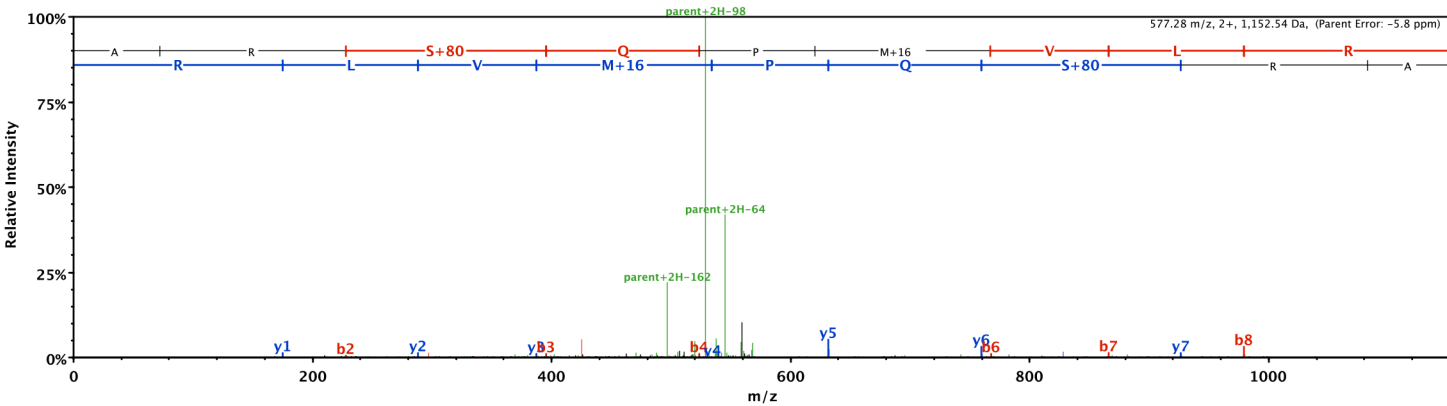
MS3



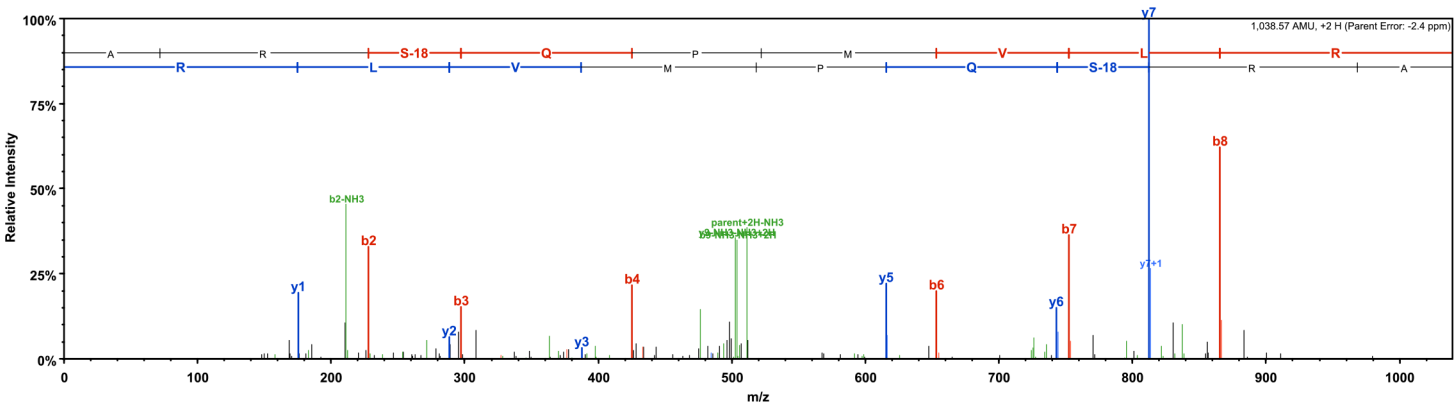


# CDK5 S728

## MS2

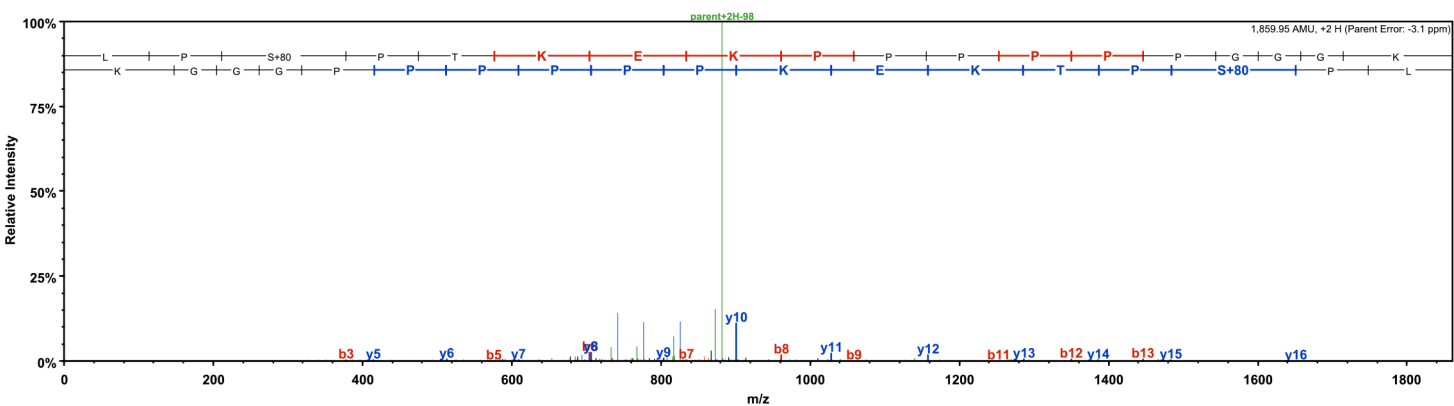


# MS3

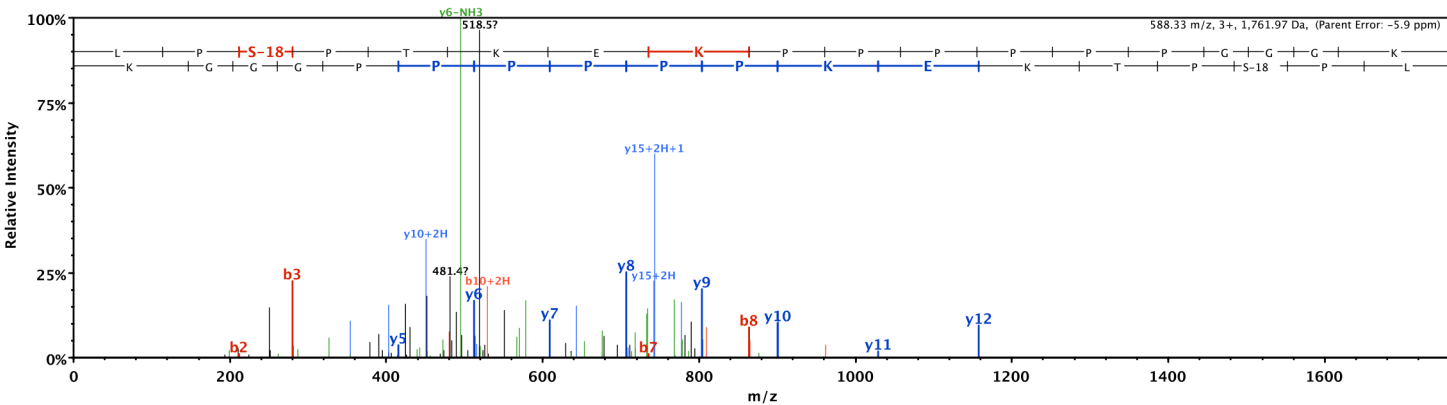


# CDK5 S773

## MS2

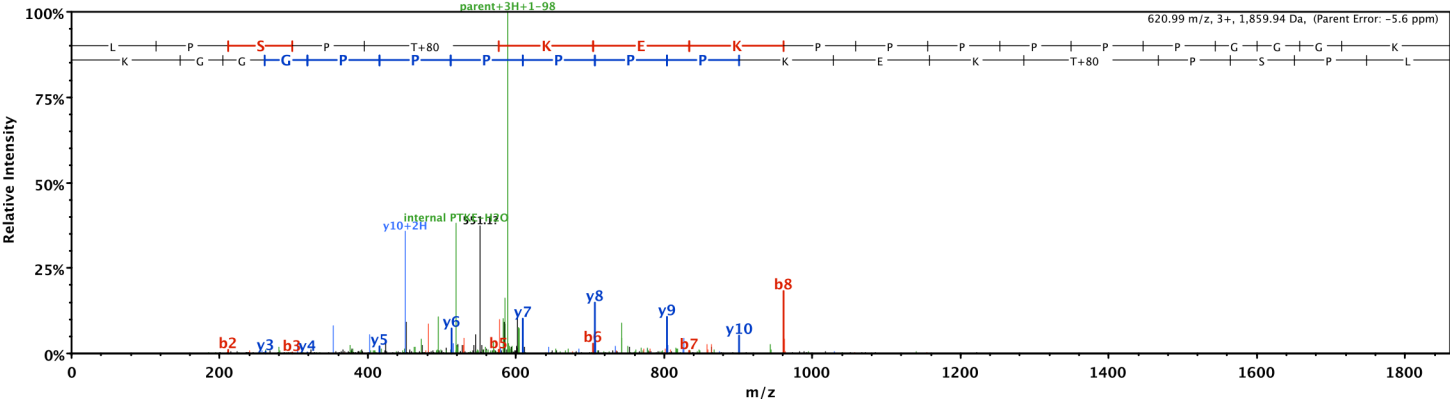


# MS3

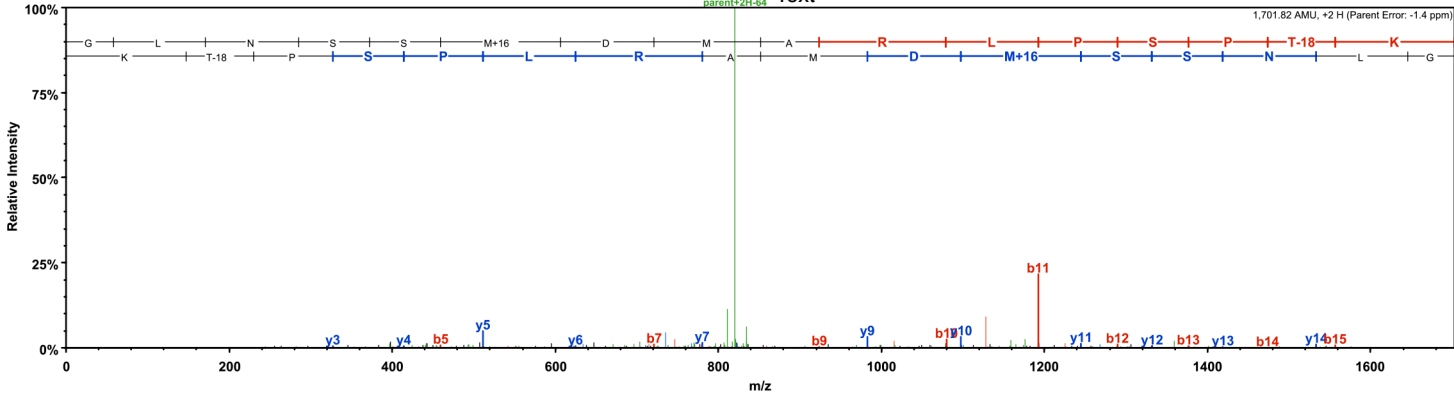


CDK5 T775

MS2

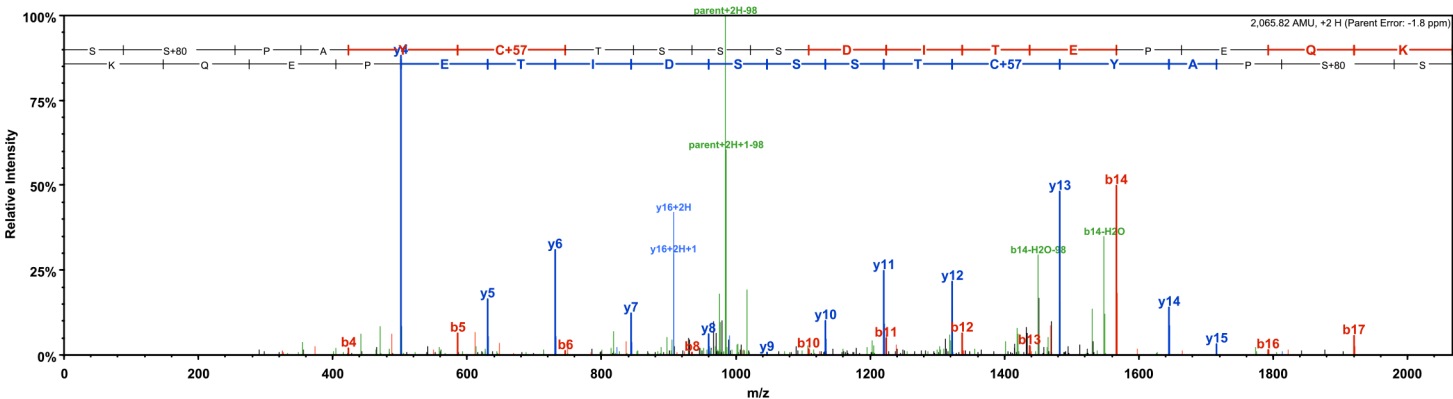


MS3

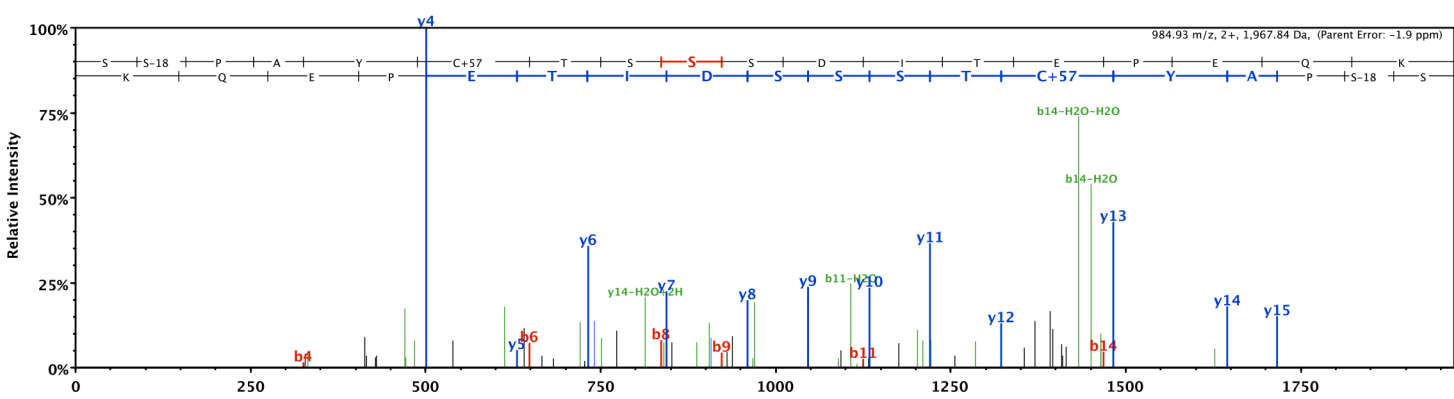


CDK5 S802

MS2



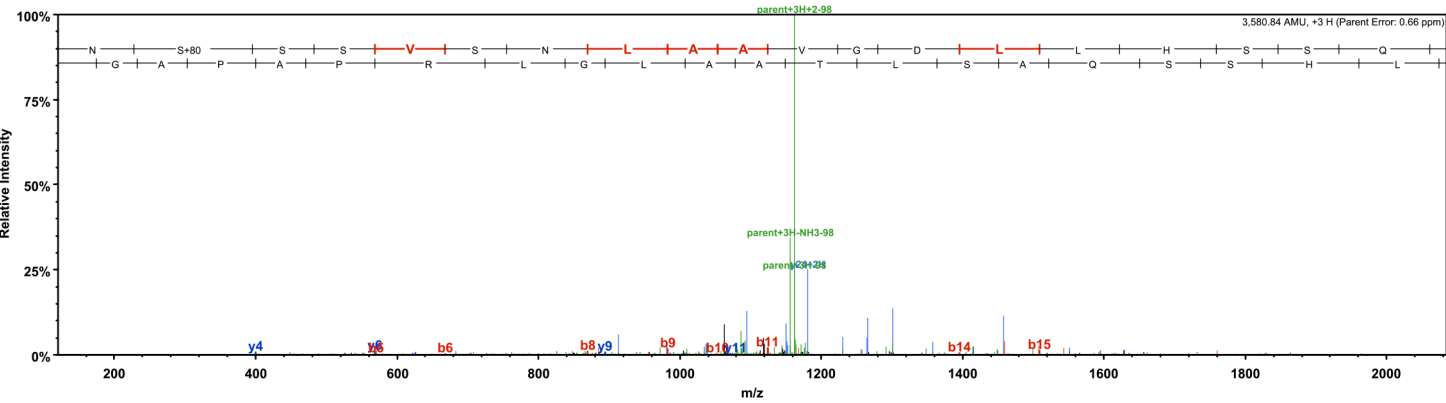
MS3





CDK5 S842

MS2



MS3

